REMARKS

Reexamination and reconsideration in light of the foregoing amendments and following remarks is respectfully requested.

I. AMENDMENTS

Claim 13 is under examination.

Claim 13 has been amended to deleted "dose" and replace it with "amount" in partial response to the Examiner's 35 U.S.C. § 112, second paragraph rejection.

The Applicants aver that the amendment does not add new subject matter and respectfully request entry of the claim.

II. THE CLAIM AS AMENDED IS PATENTABLE UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claim 13 stands rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement. The Examiner maintains that the specification does not reasonable provide enablement for the claimed bioavailable pharmaceutical composition comprising a therapeutic quantity of a COX-2 inhibitor having an IC50-WHMA COX-2/COX-1 ratio ranging from about 0.23 to about 3.33 with reduced gastrointestinal and cardiovascular toxicity as compared to the Wands factor (see In re Wands, 8 USPQ2nd 1400 (Fed. Cir., 1988) as to undue experimentation.

The Examiner further states that the "Applicants have not reasonably demonstrated/disclosed that the claimed extract composition has the claimed therapeutic quantity. There is no way for one of ordinary skill in the art to reasonably calculate if the claimed extract is enabled or not. The ratios are very ambiguous and hard to quantify against the prior art." The Applicants respectfully disagree.

The Applicants maintain that the specification and claims have been enabled as to the Wands factors in that:

a. **The nature of the invention** is directed towards a proposed medical treatment limited to osteo or rheumatoid arthritis, or pain. These conditions being common and not unique in modes of action for potential treatments. Further, the Applicants maintain that the causative etiological factors for induction or control are known to one of skill in the art (see paragraphs [0002] to [0009]).

b. **The breadth of the claims** is narrow in that it is directed to treatment of three conditions (osteo or rheumatoid arthritis, or pain) using a composition comprising a therapeutic quantity of a COX-2 inhibitor having an IC50-WHMA COX-2/COX-1 ratio ranging from about 0.23 to about 3.33 with reduced gastrointestinal and cardiovascular toxicity using a defined amount of the COX-2 inhibitor.

c. **The predictability or unpredictability of the art**, as to the conditions being treated as per the claim, are considered fairly predictable as there are numerous assays for range of movement, etc., to measure efficacy of the proposed treatment. The Applicants do concede that some responses, for example reduction in pain, may be more subjective in measuring.

d. **The amount of direction or guidance provided** is believed to be sufficient to enable on of skill in the art to practice the invention. The Applicants respectfully maintain that sufficient guidance has been provided in the disclosure as to each key element of the claims so as to enable the invention absent undue experimentation.

    1. First as to bioavailability of the composition. Here the Examiner's attention is directed to paragraph [0024] indicating that the iso-alpha acids disclosed should be more bioavailable than alpha acids.

2. Second, as to COX inhibition, the Examiner is directed to Example 4, paragraphs [0065] to [0067] for methodology as to determining COX inhibition.

3. Third, the methodology for determining the WHMA COX-2/COX-1 ratio is described in T. D. Warner et al., Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis, Proc. Natl. Sci. USA 96:7563-68 (1999), incorporated by reference in paragraph [0029] (copy provided).

4. Last, the procedures for producing iso-alpha acids by heating a standardized hops extract in aqueous solution with potassium hydroxide is disclosed in Example 7, paragraphs [071] to [0073]. Not disclosed is the amount of time for heating the extract, which the Applicants maintain does not require undue experimentation.

e. **The presence or absence of working examples** has been met in that all steps absent the heat step of d(4) above have been provided, For example, Examples 6 – 8 provide guidance for producing pharmaceutical compositions.

f. **The quantity of experimentation necessary** is considered minimal. The Applicants maintain that the specification provides all the guidance required to produce the composition for use in the method of treatment. Applicants assert that a practitioner will appreciate that the compounds, compositions, and methods described in the specification are to be used in concomitance with continuous clinical evaluations by a skilled practitioner (physician or veterinarian) to determine subsequent therapy. Hence, following treatment the practitioners will evaluate any improvement according to standard methodologies. Such evaluation will aid and inform in evaluating whether to increase, reduce or continue a particular treatment dose, mode of administration, etc. This is well within the scope of practice of one of ordinary

skill in the art, especially in light of the fact that the method prescribes the range of use of the compounds of the invention.

g. **The state of the art** and the **relative skill of those skilled in the art** are such that the invention may be practiced with ease. The production of the composition of the method requires simple medicinal chemistry to produce and, as discussed above, treatment using that composition is well within the skill of a treating clinician.

As such, the Applicant submits that the application has been enabled under 35 U.S.C. § 112, first paragraph and respectfully requests withdrawal of the rejection of Claim 13.

III. THE CLAIM AS AMENDED IS PATENTABLE UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claim 13 stands rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. The Applicant respectfully disagrees.

The Examiner maintains that it is not clear how to quantify the claim ratio for a COX-2 inhibitor having an IC50-WHMA COX-2/COX-1 ratio ranging from about 0.23 to about 3.3. As discussed above, the methodology for determining the WHMA COX-2/COX-1 ratio is described in T. D. Warner et al., Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis, Proc. Natl. Sci. USA 96:7563-68 (1999), incorporated by reference in paragraph [0029] (copy provided). As cited throughout the specification, the WHMA COX-2/COX-1 ratio ranging from about 0.23 to about 3.3 is what imparts the reduced toxicities.

As such, the Applicant submits that the application has been enabled under 35 U.S.C. § 112, second paragraph and respectfully requests withdrawal of the rejection of Claim 13.

IV. CLAIMS 13 AS AMENDED IS PATENTABLE UNDER 35 U.S.C. § 103(A)

Claims 13 stands rejected under 35 U.S.C. § 103(a) as being anticipated by Rigby et al (US 3,354,219; hereinafter "Rigby") in view of Todd, Jr et al (US 5,041,300) as evidenced by Medicinenet.com and About.com.

The Examiner states that Rigby teaches that hot water, NaOH, and hops are boiled for two hours and that the reference additionally noted that KOH can be used instead of NaOH.

The Examiner further states that Medicinenet makes it clear that acute pain comes on quickly and thus reads on anyone since anyone can have acute pain. The Examiner goes on to state that About.com makes it clear that standardized extracts have been processed to contain a specific amount of a compound but as seen in Rigby once the extract is reacted with KOH a specific amount of iso-alpha acids are formed.

The Applicant respectfully disagrees with the Examiners assertion of Claim 13, as amended, being anticipated by Rigby et al (US 3,354,219; hereinafter "Rigby") in view of Todd, Jr et al (US 5,041,300) as evidenced by Medicinenet.com and About.com.

The Applicant maintains that the reference art cited neither teaches each and every element of a claim nor provides any motivation to combine the references to produce the instant invention and is therefore not obvious.

Rigby is merely directed to a method for producing an isohumulone concentrate. Rigby neither teaches nor suggests that the isohumulone concentrate can be used to treat any condition, let alone osteoarthritis, rheumatoid arthritis, or acute pain as in the instant case. Furthermore, Rigby neither teaches nor suggests the dose of the COX-2 inhibitor having ranges from about 5 mg to about 1,000 mg per day nor defines the COX-2 inhibitor having an IC50-WHMA COX-2/COX-1 ratio ranging from about 0.23 to about 3.3.

Medicinenet.com merely provides a definition of acute pain while About.com defines a standardized extract and the Applicant maintains that neither reference corrects the deficiencies of Rigby as to currently amended Claim 13.

Additionally, Todd fails to correct these deficiencies in that Todd is directed to modification for the flavoring of beer. Todd neither teaches nor suggests that the components disclosed have reduced gastric or cardiovascular toxicity nor that the may be used for the treatment of osteo or rheumatoid arthritis, or pain or any medical condition.

Taken in their totality, the art cited should not be considered as rendering the current invention obvious in that they fail to teach or suggest that the compositions disclosed therein have and medicinal utility nor do they provide any expectation for success absent undue experimentation.

As such, the Applicant submits that Rigby in view of Todd, Jr et al (US 5,041,300) as evidenced by Medicinenet.com and About.com is not obvious to amended Claim 13 and respectfully request withdrawal of the 35 U.S.C. § 103(a) rejection of Claim 13.

VI. CONCLUSION

On the basis of the foregoing remarks and amendments, Applicants respectfully submit that amended Claims 13 is in condition for allowance. Passage to issue is respectfully requested.

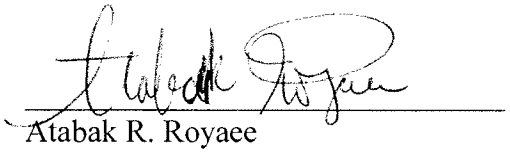
If there are any questions regarding these remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

A Request for a Three (3) Month Extension of Time, up to and including November 13, 2009 is included herewith. Pursuant to 37 C.F.R. § 1.136(a)(2), the Examiner is authorized to charge any fee under 37 C.F.R. § 1.17 applicable in this instant, as well as in future communications, to Deposit Account 50-1133.

Furthermore, such authorization should be treated in any concurrent or future reply requiring a petition for an extension of time under paragraph 1.136 for its timely submission, as constructively incorporating a petition for extension of time for the appropriate length of time pursuant 37 C.F.R. § 1.136(a)(3) regardless of whether a separate petition is included.

Respectively submitted,

MCDERMOTT WILL & EMERY LLP



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Pharmacology

Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full *in vitro* analysis

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Contributed by John R. Vane, April 14, 1999

ABSTRACT **The beneficial actions of nonsteroid anti-inflammatory drugs (NSAID) can be associated with inhibition of cyclo-oxygenase (COX)-2 whereas their harmful side effects are associated with inhibition of COX-1. Here we report data from two related assay systems, the human whole blood assay and a modified human whole blood assay (using human A549 cells as a source of COX-2). This assay we refer to as the William Harvey Modified Assay. Our aim was to make meaningful comparisons of both classical NSAIDs and newer COX-2-selective compounds. These comparisons of the actions of >40 NSAIDs and novel COX-2-selective agents, including celecoxib, rofecoxib and diisopropyl fluorophosphate, demonstrate a distribution of compound selectivities toward COX-1 that aligns with the risk of serious gastrointestinal complications. In conclusion, this full *in vitro* analysis of COX-1/2 selectivities in human tissues clearly supports the theory that inhibition of COX-1 underlies the gastrointestinal toxicity of NSAIDs in man.**

Nonsteroid anti-inflammatory drugs (NSAIDs) are among the most widely prescribed drugs worldwide, being the drugs of first choice in the treatment of rheumatic disorders and other degenerative inflammatory joint diseases. Inhibition of cyclo-oxygenase (COX), and therefore prostaglandin production, is the common mechanism of action of the NSAIDs (1). As is now well appreciated, COX exists as two isoforms. In general terms, cyclo-oxygenase-1 (COX-1) is constitutive and present in, for example, the endothelium, stomach and kidney whereas cyclo-oxygenase-2 (COX-2) is induced by proinflammatory cytokines and endotoxin in cells *in vitro* and at inflammatory sites *in vivo* (see ref. 2). This led some of us to the previous proposition that the side effects of NSAIDs correlate with their ability to inhibit COX-1 whereas the therapeutic, anti-inflammatory effects of these agents are attributable to their ability to inhibit COX-2 (3). A number of subsequent analyses have been published demonstrating the potencies against COX-1 and COX-2 of a large number of NSAIDs and novel COX-2-selective inhibitors (see ref. 2). Although these analyses have used a wide range of assay systems, from isolated purified enzymes to intact cells, the assay most widely accepted is the human whole blood assay (4–7). This assay has the advantage of using readily available human cells and taking into account the binding of NSAIDs to human plasma proteins. However, thus far, there are no single studies published that compare the relative abilities of all members of the NSAID family to inhibit COX-1 versus COX-2 on a common and appropriate assay system. Without such information, it is not possible to determine the predictive nature of such assays for the use of NSAIDs in the patient population. Here we present data derived from both the human whole blood assay (WBA) and a human modified whole blood assay (WHMA) for >40 NSAIDs and COX-2-selective inhibitors. These data support the concept that inhibition of COX-1 is responsible for the serious gastrointestinal (GI) complications induced by NSAIDs in humans (8).

METHODS

Cell Culture. Human airway epithelial cells, A549 cells (European Collection of Animal Cell Cultures, ref. no. 86012804) were cultured in 96-well plates with DMEM supplemented with 10% fetal calf serum and L-glutamine (4 mM). To induce the expression of COX-2, A549 cells were exposed to interleukin-1β (10 ng·ml^{-1}) for 24 h (9).

Human Whole Blood Assay (WBA). Blood was collected by venupuncture into heparin (19 units/ml) and then was aliquoted in 100-μl volumes into the individual wells of 96-well plates. For COX-1 assays, blood then was treated with test agents or vehicle (usually 0.1% vol/vol dimethyl sulfoxide) followed 60 min later by calcium ionophore, A23187 (50 μM). After 30 min, the plates were centrifuged (1,500 × *g*, 4°C, 5 min), and the plasma was removed and immediately frozen. For WBA COX-2 assays, blood was treated with aspirin (12 μg/ml) to inactivate COX-1, and then 6 h later with lipopolysaccharide (10 μg/ml) plus test agents or vehicle. Incubation then was continued for a further 18 h, after which time the plates were spun, and the plasma was removed and frozen. Concentrations of thromboxane (Tx) B_2 (as a measure of TxA_2 formation and so COX activity) in samples from both protocols then were determined by radioimmunoassay. Data is reported as being from COX-1 and WBA-COX-2 protocols.

William Harvey Human Modified Whole Blood Assay (WHMA). For assay of COX-1, experiments were conducted as above, and all COX-1 data were pooled. For assay of COX-2, the medium was removed from A549 cells, which had been exposed to interleukin-1β for the preceding 24 h, and human blood (100 μl) added together with test agents or vehicle. Sixty minutes later, A23187 (50 μM) was added, followed 30 min later by diclofenac (1 mM) to inhibit (>98%) the formation of prostanoids. The plates then were centrifuged, and plasma was removed (as above). Concentrations of prostaglandin E_2 (PGE_2) in samples then were determined by radioimmunoassay as a measure of the activity of COX-2 in the A549 cells. Data is reported as being from the WHMA-COX-2 protocol.

Materials. Radiolabeled [^{3}H]TxB_2 and [^{3}H]PGE_2 were obtained from Amersham. Celecoxib, L-745,337, SC58125, and rofecoxib were synthesized by Boehringer Ingelheim; 6-methoxy-2-napthylacetic acid (6MNA) was a gift from SmithKline Beecham; diisopropyl fluorophosphate was a gift from Merck-

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Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclo-oxygenase; WBA, whole blood assay; WHMA, William Harvey human modified whole blood assay; Tx, thromboxane; PGE_2, prostaglandin E_2; 6MNA, 6-methoxy-2-napthylacetic acid; GI, gastrointestinal.

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Frosst Labs (Pointe Claire, PQ, Canada); tomoxiprole was a gift from NicOx S.A. (Nice, France); ketorolac, meclofenamate, niflumic acid, NS398, and valeryl salicylate were obtained from SPI Bio (Massy Cedex, France); and sulindac sulfide was purchased from Affiniti (Exeter, U.K). All other compounds and reagents were obtained from Sigma.

Calculations. For each blood sample, the "control" formation of TxB_2 or PGE_2 was assessed as the mean of six determinations. For each experiment, the effects of the compounds were calculated and represented as percent of control by using the mean control value. Concentration response curves were fitted, and IC_{50} and IC_{80} values were derived, by using PRISM (GraphPad, San Diego). COX-1/WBA-COX-2 (WBA) and COX-1/WHMA-COX-2 (WHMA) selectivities were determined as the ratios of the IC_{50} and IC_{80} values.

RESULTS

Prostanoid Production. In the presence of drug vehicle, the productions of prostanoids in the assay systems were: COX-1, 32.3 ± 1.9 ng·ml^{-1} TxB_2; WBA-COX-2, 12 ± 0.6 ng·ml^{-1} TxB_2; and WHMA-COX-2, 41.8 ± 1.9 ng·ml^{-1} PGE_2 (n = 24–31). In blood treated with aspirin and then incubated for 18 h in the absence of lipopolysaccharide, there was no detectable formation of TxB_2 or PGE_2.

Inhibitor Potencies. The agents tested readily divided into four groups in terms of their potencies as inhibitors of COX-1 and COX-2 (Table 1; Figs. 1–4). The first group consists of compounds that can produce full inhibition of both COX-1 and COX-2 with relatively poor selectivity. This group contained most of the currently used NSAIDs, including, for instance, diclofenac, ibuprofen, naproxen, piroxicam, and sulindac (Fig. 1) as well as 6MNA, the active metabolite of nabumetone. Aspirin could not be assessed in the WBA-COX-2 assay because of its instability in whole blood but was active in the WHMA-COX-2 assay. Taken together with the COX-1 assay, our data demonstrated a selectivity of aspirin of ≈4-fold toward COX-1. The second group contained compounds such as etodolac, meloxicam, and nimesulide, all of which show a preferential selectivity toward COX-2 (>5-fold in the WHMA/COX-1 determination) (Fig. 1). It must not be overlooked, however, that these compounds all have the potential to produce full inhibition of COX-1. Of interest, our data also indicate that celecoxib should be included in this second group (Fig. 1). The third group contained compounds that inhibit COX-2 with only a very weak activity against COX-1 and included the experimental compounds diisopropyl fluorophosphate, L-745,337, NS398, and SC58125 together with rofecoxib, all of which were designed as COX-2-selective agents (Fig. 2). The fourth group contained compounds that appeared to be only weak inhibitors of COX-1 and COX-2, such as many of the salicylates. As expected, this fourth group also included nabumetone, which, unlike its metabolite 6MNA, only produced weak inhibition of both COX isoforms.

DISCUSSION

Here, using simple assay systems, we have investigated the relative potencies as inhibitors of COX-1 and COX-2 of a wide range of NSAIDs as well as representatives of the newer COX-2 selective agents. In particular, however, we also included all of those agents for which good epidemiological data of the risk of serious GI complications existed (8). This was a deliberate approach because, although some of these compounds were previously tested in other human whole blood assays (e.g., refs. 4–7), they have not been tested together within a single assay system.

When comparing the potencies of NSAIDs against COX-1 and COX-2, IC_{50} values are often used. However, there are assumptions underlying such an approach that are not necessarily correct. In particular, as is clear from Figs. 1 and 2, the inhibitor curves are often not parallel. Thus, as the concentration of a NSAID varies, so does its relative potency. Second, NSAIDs are used therapeutically at doses that produce more than a 50% reduction in prostanoid formation. Indeed, a survey of the literature established that, for diclofenac (10), etodolac (11), indomethacin (12, 13), fenoprofen (12), flurbiprofen (14), ketoprofen (12), ketorolac (13, 15), meclofenamate (12), meloxicam (16), naproxen (17), nimesulide (18), piroxicam (19), sulindac (20), and tolmetin (12), the steady-state plasma concentrations of these drugs, as well as the peak concentrations of aspirin (12), would produce average inhibitions in our assay systems of 82 ± 5% (COX-1), 74 ± 5% (WBA-COX-2), and 89 ± 2% (WHMA-COX-2) (n = 15). Comparison of the potencies of the NSAIDs against COX-1 and COX-2 at the IC_{80} value, therefore, appears more appropriate. In making these comparisons, we used data both from the WBA and from the WHMA. This second assay was developed because the potencies of NSAIDs as inhibitors of prostanoid formation are influenced by the supply of arachidonic acid both *in vitro* (21) and *in vivo* (22). Clearly, in the standard human whole blood assay, there is a substantial difference between the time courses of the incubations for testing inhibition of COX-1 and COX-2 (1 h vs. 18 h) and, hence, in the rate of prostanoid formation and so in the supply of arachidonic acid. The human whole blood plus A549 cell assay provides a system in which COX-2-containing cells are exposed to NSAIDs for the same time periods and in which the same stimulus is applied at the end of this incubation period, as for the matched COX-1 assay system. Of interest, a number of the compounds tested appeared more potent in the WHMA-COX-2 than the WBA-COX-2. This could be explained by variations in either the metabolism or the plasma binding of compounds within the blood samples during the different time courses of the WBA and WHMA. Alternatively, it could be explained by different levels or sources of free arachidonic acid within the cells expressing COX-2 in the two assay systems, or even to the binding characteristics of the NSAIDs to COX-2 (23).

When making our comparisons from the two assay systems we found that the agents tested could be divided into four main groups: (*i*) compounds capable of producing full inhibition of both COX-1 and COX-2 with poor selectivity; (*ii*) compounds capable of producing full inhibition of COX-1 and COX-2 with preference toward COX-2; (*iii*) compounds that strongly inhibited COX-2 with only weak activity against COX-1; and (*iv*) compounds that appeared to be only weak inhibitors of COX-1 and COX-2 (Table 1; Fig. 3). It is of interest to compare these groupings of NSAIDs to epidemiological studies of NSAID-induced GI toxicity. This is an area of particular interest, for NSAIDs cause serious gastric damage leading to hospitalization in some 100,000 patients per year in the U.S. alone (24). The relationship between NSAID use and serious GI complications has, therefore, been examined in a number of studies. One of the most complete recent studies is a meta-analysis of reports between 1985 and 1994 (8) in which 11 NSAIDs (plus azapropazone) were ordered for their association with serious complications. The order of the NSAIDs, from least to most damaging, was 1-ibuprofen, 2-diclofenac, 3-diflunisal, 4-fenoprofen, 5-aspirin, 6-sulindac, 7-naproxen, 8-indomethacin, 9-piroxicam, 10-ketoprofen, and 11-tolmetin, with azapropazone last. (We have not included azapropazone in any of our subsequent analyses). Group 1 (see Table 1) contained all of the NSAIDs included in this analysis. This is consistent with the idea that NSAIDs produce serious GI complications by significantly inhibiting the activity of COX. Further comparison of the COX-1 selectivities of these compounds (Fig. 3) demonstrates that compounds associated with the greatest GI toxicity have the greatest COX-1 selectivity. These include tolmetin, indomethacin, ketoprofen (8), and, in particular, ketorolac. It is notable that we found ketorolac to be the most COX-1 selective of all of the NSAIDs we tested because this compound is ≈5× more gastrotoxic than other NSAIDs (25). Clearly, this is in keeping with the idea that COX-1 inhibition underlies the serious GI complications of NSAIDs; ketorolac is an extreme outlier both in our assay system and in epidemiological reports.

Table 1. Potencies of all compounds tested as inhibitors of prostanoid formation determined in the COX-1 assay, WBA-COX-2, and WHMA-COX-2

	COX-1		WBA-COX-2		WHMA-COX-2		IC_{50} ratios		IC_{80} ratios		Ranking at IC_{80} ratios	
Compound	IC_{50}, μM	IC_{80}, μM	IC_{50}, μM	IC_{80}, μM	IC_{50}, μM	IC_{80}, μM	WBA COX-1	WHMA COX-1	WBA COX-1	WHMA COX-1	WBA COX-1	WHMA COX-1
6MNA	42	130	146	580	n.d.	n.d.	3.5	n.d.	4.5	n.d.	27	n.d.
Aspirin	1.7	8.0	>100	>100	7.5	30	>100	4.4	>100	3.8	34	23
Carprofen	0.087	19	4.3	75	n.d.	n.d.	50	n.d.	3.9	n.d.	25	n.d.
Diclofenac	0.075	1.0	0.038	0.27	0.020	0.23	0.5	0.3	0.27	0.23	10	9
Fenoprofen	3.4	23	41	100	5.9	24	12	1.7	4.3	1.0	26	18
Flufenamate	3.0	80	9.3	79	n.d.	n.d.	3.1	n.d.	1.0	n.d.	13	n.d.
Flubiprofen	0.075	1.0	5.5	24	0.77	51	73	10	24	51	31	27
Ibuprofen	7.6	58	7.2	67	20	150	0.9	2.6	1.2	2.6	14	20
Indomethacin	0.013	0.46	1.0	5.0	0.13	2.0	80	10	11	4.3	29	24
Ketoprofen	0.047	1.0	2.9	22	0.24	6.0	61	5.1	22	6.0	31	25
Ketorolac	0.00019	0.0034	0.086	4.0	0.075	1.0	453	395	1176	294	33	28
Meclofenamate	0.22	3.0	0.7	8.0	0.2	1.0	3.2	0.91	2.7	0.3	22	11
Mefenamic acid	25	>100	2.9	>100	1.3	>100	0.11	0.049	-	-	-	-
Naproxen	9.3	110	28	260	35	330	3.0	3.8	2.4	3.0	18	22
Niflumic acid	25	77	5.4	35	11	74	0.22	0.43	0.45	1.0	12	16
Piroxicam	2.4	15	7.9	31	0.17	7.0	3.3	0.1	2.1	0.47	17	13
Sulindac sulphide	1.9	38	55	100	1.21	11	29	0.64	2.6	0.29	20	10
Suprofen	1.1	3.0	8.7	56	8.3	100	7.7	7.3	19	33	30	26
Tenidap	0.081	5.0	2.9	13	n.d.	n.d.	35.2	n.d.	2.6	n.d.	21	n.d.
Tolmetin	0.35	5.0	0.82	43	1.3	13	2.3	3.8	8.6	2.6	28	21
Tomoxiprol	7.6	35	20	84	0.32	13	2.7	0.042	2.4	0.37	19	12
Zomepirac	0.43	2.0	0.81	6.0	0.096	2.0	1.9	0.22	3.0	1.0	23	17
Celexocib	1.2	28	0.83	6.0	0.34	3.0	0.7	0.3	0.21	0.11	8	7
Etodolac	12	69	2.2	8.0	0.94	3.0	0.2	0.1	0.12	0.043	6	5
Meloxicam	5.7	22	2.1	7	0.23	2.0	0.37	0.040	0.32	0.091	11	6
Nimesulide	10	41	1.9	7.0	0.39	7.0	0.19	0.038	0.17	0.17	7	8
Diisopropyl fluorophosphate	>100	>100	0.76	4.0	0.17	5.0	<0.01	<0.01	<0.01	<0.01	1=	1=
L745,337	>100	>100	8.6	41	1.3	17	<0.01	<0.01	<0.01	<0.01	1=	1=
NS398	6.9	65	0.35	1.0	0.042	1.0	0.051	0.0061	0.015	0.015	5	4
Rofecoxib	63	>100	0.84	6.0	0.31	5.0	0.013	0.0049	<0.05	<0.05	4	3
SC58125	>100	>100	2.0	10	n.d.	n.d.	>0.01	n.d.	<0.01	n.d.	1=	n.d.
5-Aminosalicylic acid	410	>1000	61	>1000	n.d.	n.d.	0.15	n.d.	-	n..d.	-	n.d.
Ampyrone	55	270	203	1000	85	670	3.7	1.5	3.7	2.5	24	19
Diflunisal	113	530	8.2	140	134	400	0.1	1.2	0.26	0.75	9	14
Nabumetone	460	>1000	>1000	>1000	290	>1000	-	-	-	-	-	-
Paracetamol	>100	>100	49	>100	64	>100	-	-	-	-	-	-
Resveratrol	30	>100	39	>100	n.d.	n.d.	1.3	n.d.	-	-	-	n.d.
Salicin	>100	>100	>100	>100	n.d.	n.d.	-	n.d.	-	-	-	n.d.
Salicylaldehyde	>100	>100	>100	>100	n.d.	n.d.	-	n.d.	-	-	-	n.d.
Sodium salicylate	4956	49000	34440	101000	482	45000	6.9	0.10	2.1	0.92	16	15
Sulfasalazine	3242	6400	2507	8300	n.d.	n.d.	0.8	n.d.	1.3	n.d.	15	n.d.
Sulindac	>100	>100	>100	>100	58	>100	-	-	-	-	-	-
Tamoxifen	15	>100	95	>100	n.d.	n.d.	6.4	n.d.	-	-	-	n.d.
Ticlopidine	52	>100	47	>100	n.d.	n.d.	0.9	n.d.	-	-	-	n.d.
Valeryl salicylate	42	>100	2.3	>100	n.d.	n.d.	0.053	n.d.	-	n.d.	-	n.d.

Data is presented in the following column order: alphabetical listing of agents after division into four main groups: (top) compounds that can produce full inhibition of both COX-1 and COX-2 with poor COX-2 selectivity; (second) compounds that can produce full inhibition of COX-1 and COX-2 with >5× preference towards inhibiting COX-2 (WHMA/COX-1 < 0.2); (third) compounds that appear to be only weak inhibitors of COX-1 and COX-2. Shown are potencies (micromolar IC_{50} and IC_{80} values) of compounds against COX-1, WBA-COX-2, and WHMA-COX-2. Selectivities of compounds towards COX-1 were determined as IC_{50} and IC_{80} ratios for both WBA-COX-2/COX-1 and WHMA-COX-2/COX-1. Ranking of compounds as inhibitors of COX-2 relative to COX-1 are based on ordering of IC_{80} ratios; higher ranking numbers are associated with increased selectivity towards COX-1. n.d., not done.

Because all of the compounds contained within group 1 have the potential to produce full inhibition of both COX-1 and COX-2, their associated risk of producing GI toxicity can be strongly influenced by dose. This can be readily appreciated by reference to Fig. 4. Here, we have displayed the extent of COX-1 inhibition produced by individual NSAIDs at concentrations that cause 80% inhibition of COX-2. This analysis essentially provides the answer to the important question, If a NSAID is used at levels sufficient to inhibit COX-2 by 80%, i.e., to produce some therapeutic effect, by how much will COX-1 be inhibited? As can be seen, the classical NSAIDs produce inhibitions of ≈80% or more. This implies that, even for a drug such as diclofenac, which is >4-fold selective for COX-2 in terms of IC_{80} values, therapeutically relevant selectivity will be very difficult to achieve; i.e., the concentration of diclofenac necessary to produce 80% inhibition of COX-2 will produce almost 70% inhibition of COX-1. To extend this line of reasoning, it is also clear that, when relative selectivities differ by only slight amounts, other variables, such as ingested dose and plasma half-life, will have a particular influence on NSAID toxicity (26). This may well be especially true for piroxicam, which we did not find in our assays to be notably COX-1-selective despite its well established GI toxicity. Piroxi-

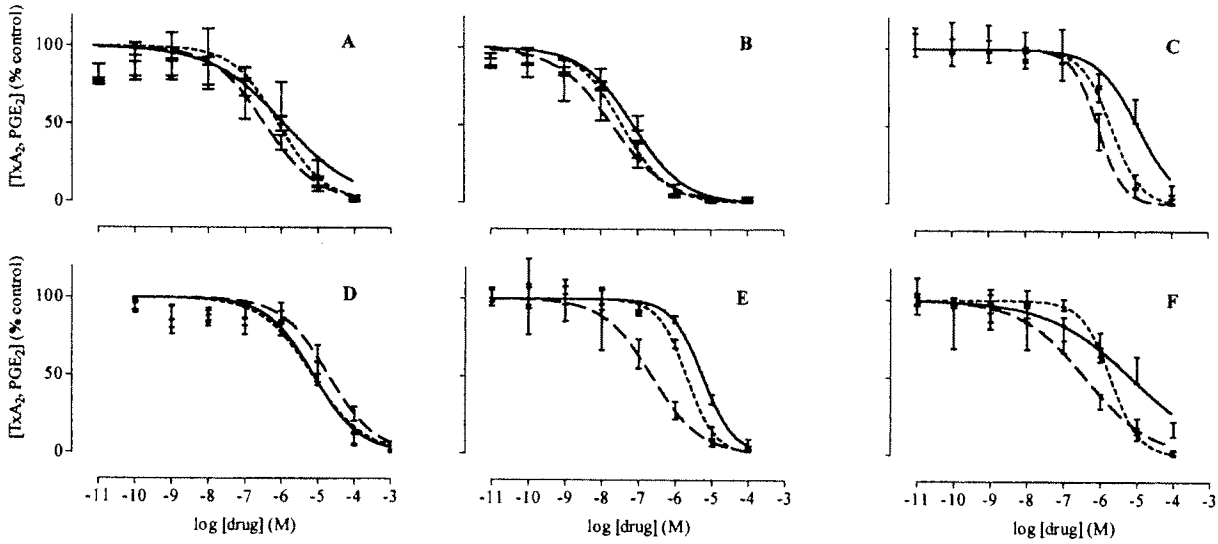

FIG. 1. The effects of celecoxib (*A*), diclofenac (*B*), etodolac (*C*), ibuprofen (*D*), meloxicam (*E*), and nimesulide (*F*) on the activity of COX-1 (solid line), WBA-COX-2 (short dashed line), and WHMA-COX-2 (long dashed line). Results are expressed as percent of control and are represented as mean ± SEM. (n = 5–8).

cam, however, has a much longer elimination half-life (30 to 70 h) (19) than other NSAIDs, and plasma half-life has been previously correlated with GI toxicity (27).

The second grouping of NSAIDs consists of preferential COX-2 inhibitors. In Fig. 3, we have classified these as compounds with between 5- and 50-fold selectivity for COX-2 over COX-1. Possibly more importantly, Fig. 4 implies that the selectivity of these compounds could be usefully exploited. For example, the concentrations of etodolac and meloxicam sufficient to inhibit COX-2 by 80% produce only 25% inhibition of COX-1. Despite the sparse epidemiological data, controlled trials [e.g., for meloxicam (28, 29)] show that these preferential compounds have an improved GI toxicity profile. It must be remembered, however, that increasing the dosage of these agents could readily increase GI toxicity due to inhibition of COX-1 because all of the compounds in this group are capable of inhibiting this isoform of COX (Fig. 1).

It is interesting that, in our assays, celecoxib was found to be a member of the preferential group of COX-2 inhibitors. This is in contrast to data derived by using recombinant human COX-1 and COX-2 from broken insect cells. In this system, celecoxib is between 155- and 3,200-fold selective for COX-2 over COX-1 (23). This difference may be attributable to the fact that celecoxib inhibition of both COX-1 and COX-2 is initially competitive with respect to substrate and is characterized by similar affinity for COX-1 and COX-2. There is a second, slow, time-dependent binding of celecoxib to COX-2 but not COX-1 that may well produce the selectivity seen in other assay systems (23). It is currently not clear why celecoxib does not demonstrate such selectivity in either the WBA or WHMA. It is unlikely that these assay systems in some way delay the time-dependent binding of celecoxib to COX-2. For instance, in the isolated human enzyme assays, this secondary binding takes place in seconds rather than minutes (23), and the WHMA assay included a preincubation period of 60 min, and the WBA included a 24-h incubation period.

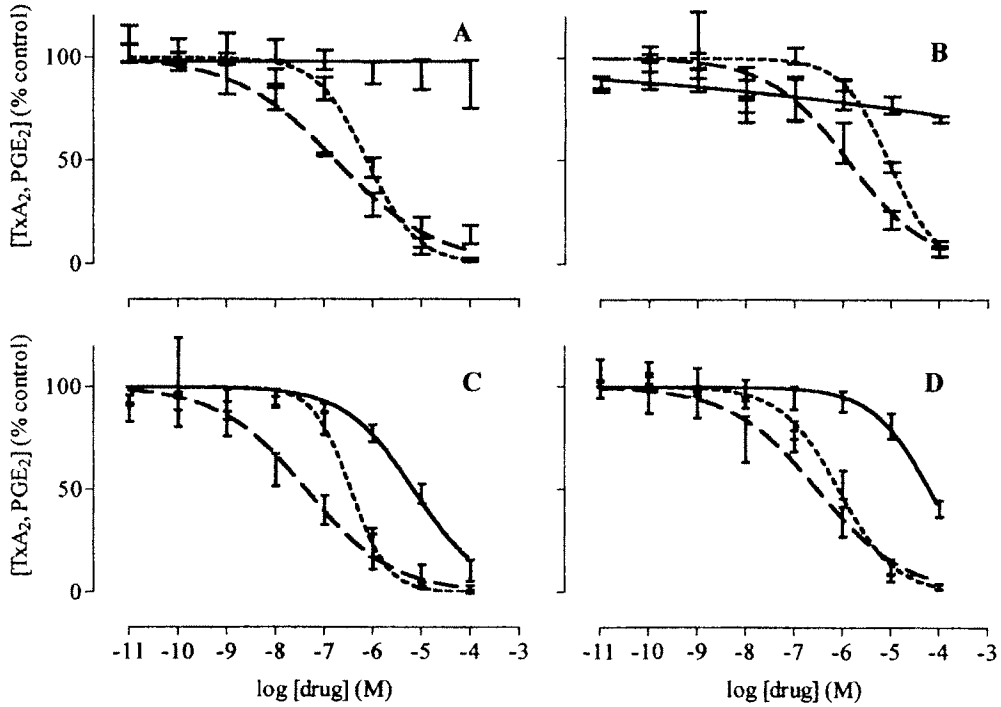

FIG. 2. The effects of diisopropyl fluorophosphate (*A*), L-745,337 (*B*), NS398 (*C*), and rofecoxib (*D*) on the activity of COX-1 (solid line), WBA-COX-2 (short dashed line), and WHMA-COX-2 (long dashed line). Results are expressed as percent of control and are represented as mean ± SEM. (n = 5–8).

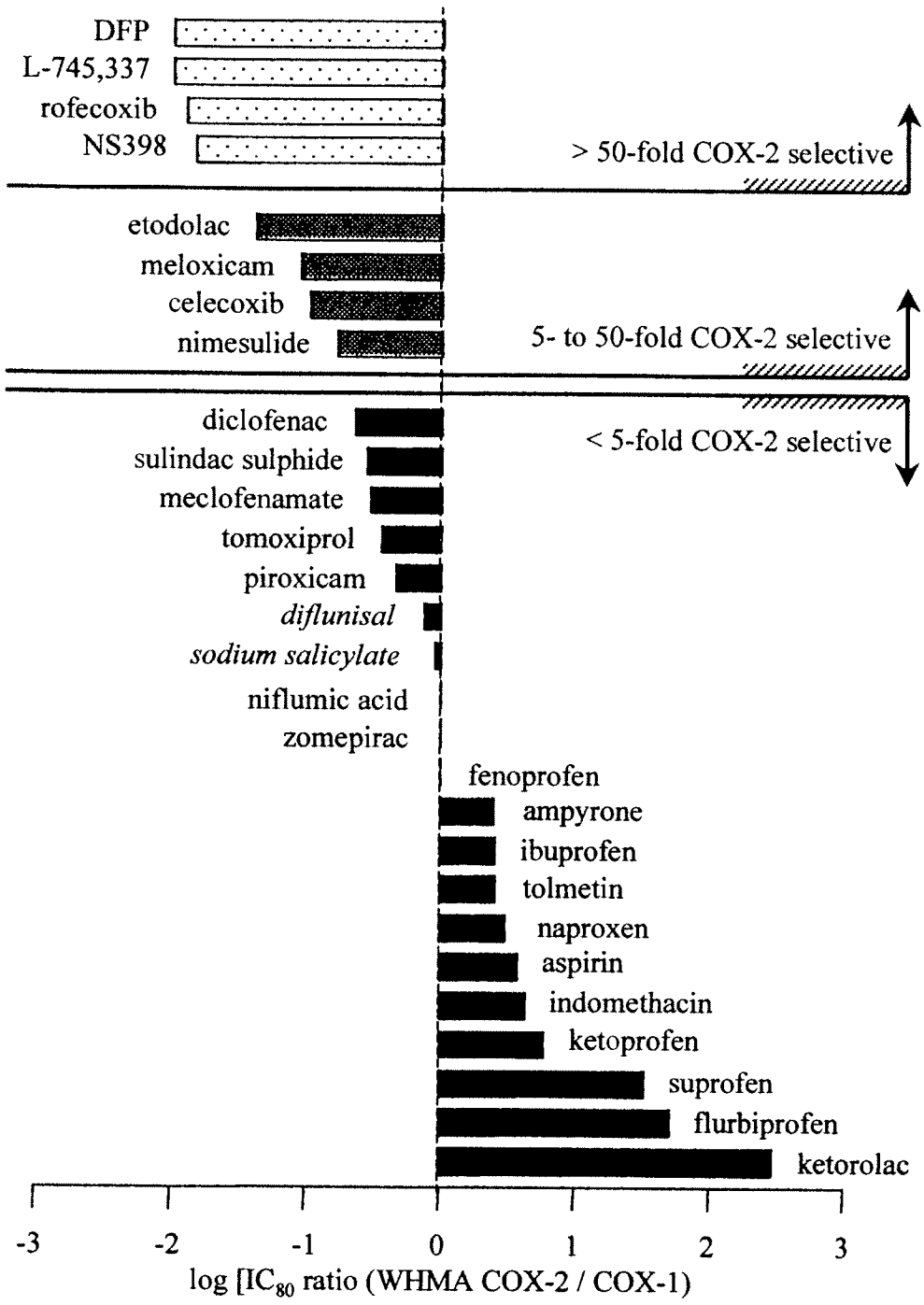

FIG. 3. Determinable log [IC_{80} ratio (WBA-COX-2/COX-1)] for all agents assayed (see Table 1). The "0 line" indicates equipotency, i.e., an IC_{80} ratio of 1. Italics indicate compounds with very low potency.

Our data also reinforce the concept that compounds within group 3 that inhibit COX-2 with only a very weak activity against COX-1 will produce few serious GI complications when used in the general population. As is clear from both the direct inhibitor curves (Fig. 2) and the derived data (Figs. 3 and 4), these compounds produce very little effect on COX-1 and should have a large therapeutic window. There are preliminary reports that rofecoxib has a low GI toxicity, but, until appropriate comparative clinical trials have been completed, no firm conclusions can be drawn (30). Furthermore, it must be remembered that studies in animals (31) suggest that when used in the presence of existing GI damage, COX-2-selective inhibitors might slow the repair process in man due to reductions in the production of protective COX-2 products (32).

Group 4 contains weak inhibitors of COX-1 and COX-2 for which reliable data with regard to inhibition of COX-1 and COX-2 could not be derived. These compounds are not, therefore, displayed in Figs. 3 and 4. Clearly, however, the weak ability of the group 4 compounds to inhibit prostanoid production explains their general lack of, or very low, GI toxicity. Sodium salicylate, for example, only caused inhibition of prostanoid formation at concentrations far in excess of those achieved *in vivo* (13) and in accordance with its relatively low GI toxicity (33). As expected, this fourth group also contained nabumetone whereas its active metabolite, 6MNA (34), was a member of the first group. This classification is in accordance with the results of Patrigiani *et al.* (4) who found that oral dosing of nabumetone at 1 g per day for 7 days reduced COX-1 activity in the WBA by 70%. The plasma concentration of drug achieved with such dosing (34) would correlate with the activity of 6MNA but not nabumetone, which we report here. As a cautionary remark to other investigators, we would like to note that we also tested six additional

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